2601-Pos Board B31
Prediction of Co-Translational Protein Folding in Living Cells
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Protein folding, the self-assembly of a protein molecule or domain into a tertiary structure, can occur as a protein molecule is being synthesized by the ribosome in a process referred to as co-translational folding. The most convincing demonstration that co-translational folding occurs inside cells comes from pulse-chase experiments in which the synthesis of the cytosolic Semliki Forest virus protein (SFVP) was monitored in Chinese hamster ovarian cells [1]. SFVP is composed of four distinct protein segments, including an N-terminal protease segment (referred to as ''C protein'') that auto-catalytically cleaves itself from the SFVP molecule once folded. The pulse-chase experiment revealed that the cleaved C protein appeared well before synthesis of the full-length SFVP was completed, demonstrating that C protein does indeed fold co-translationally in vivo. Here, we show that the time course of such co-translational folding can be accurately predicted by a chemical kinetic model using a domain's bulk folding and unfolding rates and the average rate at which codons are translated in vivo; such quantities have been reported in the literature for a number of different proteins and cell types, suggesting this theoretical approach can be applied to a wide variety of proteins. The model explains the essential features of co-translational folding time courses, and provides a means for predicting how varying the translation rate at different codon positions along a transcript's open reading frame affects this self-assembly process in vivo.
[1] Nicola, A. V., Chen, W. & Helenius, A. Co-translational folding of an alphavirus capsid protein in the cytosol of living cells. Nat. Cell Biol. 1, 341-5 (1999).

2602-Pos Board B32
Elucidating the Folding Pathways of Calcium-Binding Proteins
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During translation, ribosomes synthesize proteins according to the messenger RNA template. The polypeptide chain, specified by the template, acquires its three dimensional structure either co- or post-translationally in a process termed ''folding''. Understanding folding mechanisms is important since protein structure is critical for biological function, and misfolded proteins are correlated with cell stress and disease. Here, we map the folding pathways of two distantly related proteins: Calmodulin, a eukaryotic, calcium-dependent signaling protein, and Calerythrin, a prokaryotic, calcium-buffering protein. These proteins have a highly conserved sequence and structure dictated by their similar calcium-binding function. Both proteins have two domains, and each domain is composed of two ''EF-hand'' motifs. To probe the folding of these proteins, we utilize a focused laser beam to form an optical trap and exert mechanical force on the molecule, while measuring the molecule's response to force via its change in extension. These single-molecule experiments reveal the folding dynamics at a level of detail not possible by traditional ensemble methods, since we can directly observe folding intermediates and off-pathway states. We find that domain proximity, in this case determined by the length of a bridging helix, impacts folding and unfolding cooperativity even though the proteins share folding motifs. Studying these folding mechanisms allows us to obtain a better understanding of how domains communicate with each other and how tertiary contacts affect protein stability, while prompting further studies to examine their structure-function relationship.

2603-Pos Board B33
The Computational Studies of Co-Translational Protein Folding
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Protein synthesis in all kingdoms of life occurs on the ribosome, a macromolecular machine of >2.5 MDa. Most of the current knowledge about the crucial process of protein folding is based on in vitro investigation of isolated polypeptide chains, which typically consider the refolding of full length proteins previously denaturated by various chemical or thermal conditions. However, in vitro folding is likely to differ from in vivo, as in the latter proteins start to fold while they are still gradually emerging through the ribosomal exit tunnel. The available conformational space to the nascent polypeptide chain is therefore different from that of the full-length protein.
Excellent system to study impact of protein vectorial synthesis on protein structure and dynamics are protein C-terminal truncations. Specifically, carried in our group NMR and computational investigations of C-terminal truncations (delta4 and delta6) of an immunoglobulin fold - ddFLN, the F-actin cross-linking gelation factor from Dictyostelium discoideum - are allowing us to present, the energy landscape that emerges from these co-translational folding mimetics In my work I provide atomistic details to this energy landscape by carrying out bias exchange metadynamics simulations with chemical shifts used as structural restrains. Using this approach I overcome, in large part, the two main limitations of molecular dynamics simulations in structural studies of proteins, firstly the inaccuracies in the use of force field alone and secondly the limitations inherent in sampling of conformational space.
My study presents a structural and dynamical characterization of the free energy landscape of this C-terminal truncation as well as changes in the landscape, while a protein is folding in the vectorial manner. Hence, it is providing insights into a better understanding of co-translational folding, which still represents a major open problem in molecular biology.

2604-Pos Board B34
Rare Example of a Protein where an Isolated Domain is More Stable than the Full-Length
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Studying the contribution of individual domains to protein structure and function is of considerable interest over the years. Domains are in general less stable than the corresponding full-length proteins. Here, we report an exceptional case of utrophin tandem calponin-homology (CH) domain. Isolated C-terminal CH domain (CH2) is both thermodynamically and kinetically more stable than the full-length tandem CH domain. Reversible, equilibrium denaturant melts using both circular dichroism and protein fluorescence signals show that the CH2 is thermodynamically more stable by 4.0 kcal/mol when compared with the full-length tandem CH domain. Thermal melts indicate that CH2 unfolds at a higher temperature (15°C) than the full-length protein. Stopped-flow kinetics indicate that the CH2 unfolds slower (by 3 times) and folds faster (by 7 times) than the full-length protein, suggesting the higher kinetic stability of CH2. Analytical ultracentrifugation, size-exclusion chromatography, and dynamic light scattering show that both CH2 and the full-length protein are monomers in solution, confirming that the higher stability of CH2 is not due to formation of oligomers. Thus, the utrophin tandem CH domain is a rare example in which an isolated domain is more stable than the corresponding full-length protein.

2605-Pos Board B35
Global Contacts Direct Hydophobic Collapse in Protein Folding
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Protein folding research aims to decipher how the amino acid sequence of a protein encodes its ordered or intrinsically disordered three-dimensional structure. To address how proteins fold, how misfolding occurs, and how potential misfolding is avoided by natural proteins, we study the helical colicin immunity protein 9 (Im9) as a model system. Im9 has been characterized extensively by biochemical and molecular simulation techniques. Here, we employ a systematic computational approach to estimate the free energies of native and non-native arrangements of Im9 helices. We find that the two helical pairs that form the largest contact surfaces in the native state (helices 1 and 2, and 1 and 4) both associate in moderately non-native configurations in isolation. Our results suggest that helix 3 can assist the native docking of helix 1, and that non-helical residues on either end of helix 4 can do so even more extensively. Our results also indicate that these non-native orientations of helix 1 can rapidly relax into the native state after the native assembly of other structural components. Notably, the quantitative atomistic descriptions of transient non-native interactions derived from our studies point us beyond the simple notion that pairs of nonpolar residues can always form strong contacts, and emphasize the dependence of contact stability on the local and global environmental context.

2606-Pos Board B36
Chemical Regulation of Disulfide Coupled Folding of Disulfide Rich Peptide, Hepcidin, and its Precursor Protein
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Multi disulfide-containing-proteins are folded into the native conformations via the assistance of redox molecules, such as glutathione and cysteine in vivo. Because of this, the glutathione redox system is also generally used in in vitro folding studies [1]. However, in vitro disulfide-coupled peptide and protein folding are often problematic, since the folding intermediates are sometimes